

## Validation of Serological Correlate of Protection for Meningococcal C Conjugate Vaccine by Using Efficacy Estimates from Postlicensure Surveillance in England

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Meningococcal C conjugate (MCC) vaccines were licensed on the basis of serological correlates of protection without efficacy data. The original correlate of protection was established by using a serum bactericidal antibody assay (SBA) with human complement (hSBA), with titers  $\geq 4$  predicting protection. However, the antibody data supporting licensure were largely generated by SBA with rabbit complement (rSBA), which gives higher titers than hSBA. While rSBA titers  $\geq 128$  reliably predict protection, as measured by hSBA, sera with rSBA titers in the range of 8 to 64 may not have hSBA titers  $\geq 4$ . For rSBA titers in this equivocal range, a fourfold rise pre- to postvaccination with the MCC vaccine and/or a characteristic booster response to a polysaccharide challenge was proposed as a correlate of protection. To validate this proposed rSBA correlate, age-specific efficacy estimates for MCC vaccines obtained from postlicensure surveillance in England were compared with the efficacy predicted by the percentage of individuals in these age groups with rSBA titers above different cutoffs at 4 weeks and at 7 to 9 months after vaccination with the MCC vaccine. The average time since vaccination in the cohorts in whom efficacy was measured ranged from 8 to 10 months. The rSBA cutoff of  $\geq 128$  was shown to significantly underestimate efficacy, with rSBA cutoffs of  $\geq 4$  or  $\geq 8$  at 4 weeks postvaccination with the MCC vaccine being the most consistent with observed efficacy. When the levels obtained 7 to 9 months postvaccination with the MCC vaccine were used, all rSBA cutoffs significantly underestimated efficacy, suggesting that continuing protection is less dependent on the SBA level at the time of exposure but is more reliant on immunologic memory.

Meningococcal C conjugate (MCC) vaccines have been shown to be highly immunogenic, eliciting functional antibodies in all age groups, as measured by the serum bactericidal antibody assay (SBA). MCC vaccines were introduced in the United Kingdom in November 1999 following a decision by the United Kingdom Medicines Control Agency that, subject to adequate immunogenicity data, efficacy trials would not be required for licensure but instead serological correlates by SBAs could be relied upon (12). Studies during the 1960s with military recruits had shown that those with naturally acquired SBA titers of  $\geq 4$  were protected from meningococcal serogroup C (Men C) disease (9).

The original serological correlate of protection in military recruits was obtained by an SBA in which human serum was the exogenous complement source (hSBA). However, due to difficulties with availability, 3- to 4-week old baby rabbit serum is now recommended as an alternative complement source for SBA (11). It is generally accepted, however, that Men C organisms are more susceptible to serogroup C-specific antibodies when baby rabbit complement instead of human complement is used, resulting in higher SBA titers (10).

In the United Kingdom, correlates of protection for MCC vaccines have been reevaluated with the titers generated by

SBA with baby rabbit complement (rSBA) by using hSBA as the “gold standard” comparison (2). This showed that rSBA titers  $< 8$  predicted susceptibility and rSBA titers  $\geq 128$  predicted protection, as measured by hSBA. The main uncertainty was therefore interpretation of rSBA titers between 8 and 64, for which it was proposed that additional serological criteria would be required for the presumption of protection, namely, a fourfold rise in rSBA titer and/or demonstration of immunologic memory, as evidenced by a typical booster response to a polysaccharide challenge and immunoglobulin G avidity maturation (2). The main group of vaccinated individuals in whom substantial proportions had rSBA titers in the range of 8 to 64 were toddlers aged 12 to 14 months and, to a lesser extent, preschool children aged 3 to 4 years. Both groups received a single dose of MCC vaccine as part of the national catch-up program (12). However, almost all toddlers with postvaccination titers by rSBA in the equivocal range of 8 to 64 met the additional serological criteria required for presumption of protection (2).

The enhanced MCC surveillance program established in November 1999 (12) now allows these proposed rSBA correlates of protection to be validated against the efficacy estimates obtained for MCC vaccines from postlicensure surveillance. By using age-specific vaccine efficacy estimates and the percentage of vaccinated and unvaccinated individuals in different age groups with rSBA levels above different cutoffs, we have further investigated which cutoff by rSBA is the best predictor of protection for MCC vaccines.

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## MATERIALS AND METHODS

In order to assess the predictive value of different cutoffs by SBA as correlates of protection, it is assumed that individuals with SBA titers greater than or equal to a given cutoff are protected when they are exposed to the pathogen, whereas those with titers less than the cutoff have a probability ( $d$ ) of disease when they are exposed to the pathogen. It is also assumed that individuals may have protective titers generated by either vaccination or natural exposure and that the protection afforded at the same cutoff in both groups is equivalent. Vaccine efficacy (VE) is defined as the percent reduction of disease in vaccinated individuals compared to the incidence in unvaccinated individuals, that is,  $VE = 1 - (ARV/ARU)$ , where ARU is the attack rate in unvaccinated individuals and ARV is the attack rate in vaccinated individuals.

If it is assumed that exposure is equal in vaccinated and unvaccinated individuals, then

$$VE = 1 - \frac{d \times P(SBA_v < CO)}{d \times P(SBA_u < CO)} = 1 - \frac{P(SBA_v < CO)}{P(SBA_u < CO)}$$

where  $P(SBA_v < CO)$  and  $P(SBA_u < CO)$  indicate the probabilities that vaccinated and unvaccinated individuals have SBA titers less than the protective cutoff (CO), respectively.

From data on the SBA titers in vaccinated and unvaccinated individuals,  $P(SBA_v < CO)$  and  $P(SBA_u < CO)$  can be estimated by using the observed proportions less than the cutoffs. Therefore, we can estimate vaccine efficacy as  $1 - (\text{percent } SBA_v < CO / \text{percent } SBA_u < CO)$ . Confidence intervals for the estimated vaccine efficacy were obtained by using Taylor series expansions for confidence intervals on ratios.

The predicted vaccine efficacy estimates for various cutoffs as measured by rSBA were then compared with the observed efficacy and its 95% confidence intervals (CIs), calculated by the screening method (7). This method uses the proportion of cases vaccinated (PCV) and proportion of the population vaccinated (PPV) to estimate vaccine efficacy by the formula  $1 - \{[PCV(1 - PPV)] / [(1 - PCV)/PPV]\}$ .

Details on the use of this method for estimation of the efficacy of the meningococcal conjugate vaccine in toddlers can be found elsewhere (13). If the predicted efficacy for a given cutoff is not consistent with the observed efficacy, the cutoff cannot be used to predict protection. If the predicted vaccine efficacy for a given cutoff is consistent with the observed efficacy, this cutoff may be the valid cutoff for protection.

The significance of differences between the observed efficacy and the expected efficacy estimates is assessed by assuming that the logarithms of 1 minus the efficacy estimates are normally distributed and by using a Z test.

Observed efficacy estimates are based on the cases of confirmed Men C infection that occurred in vaccinated and unvaccinated individuals in England from January 2000 to the end of 2001. Toddlers were mostly vaccinated between January and June 2000, so efficacy estimates cover postvaccination periods up to 2 years, with an average of about 10 months. Vaccination of the preschool children cohort started in April 2000 and was completed at about the end 2000, with an average time since vaccination of 7.5 months. Infant vaccination started in November 1999 and is ongoing as children are born; efficacy estimates therefore cover postvaccination periods up to 2 years 2 months, but the average time since vaccination is less, at about 8 months.

TABLE 1. Distribution of rSBA titers after vaccination with MCC vaccine by age when vaccination was completed and time since vaccination

SBA titer	No. of individuals at the indicated times postvaccination:				
	Infants (age, 4–5 mo)		Toddlers (age, 12–14 mo)		Preschool children (age, 3–4 yr), 1 mo
	1 mo	9 mo	1 mo	7 mo	
<4	0	13	3	10	0
4	1	10	3	6	0
8	0	3	3	0	3
16	0	3	3	6	2
32	0	4	7	9	1
64	1	3	4	3	6
128+	51	14	47	31	110
Total	53	50	70	65	122

TABLE 2. Distribution of rSBA titers in an unvaccinated population

SBA titer	No. of individuals		
	Infants (age, 3–11 mo)	Toddlers (age, 12–23 mo)	Preschool children (age, 2–4 yr)
<4	142	225	189
4	6	8	6
8	5	11	2
16	5	4	2
32	2	6	4
64	3	4	1
128+	6	9	5
Total	169	267	209

The distributions of rSBA titers post-primary vaccination against MCC in infants, toddlers, and preschool children (Table 1) were taken from a series of studies (16, 14, 4) in which testing was carried out at the Manchester Public Health Laboratory by standardized rSBA methods (3). All the children received Meningitec, a cross-reactive mutant-based conjugate vaccine produced by Wyeth Lederle Vaccines (Pearl River, N.Y.). Infants were given three doses at 2, 3, and 4 months of age; and toddlers and preschool children received a single dose. At 1 month after vaccination, a blood sample was taken from all children; for infants and toddlers, late blood samples were also obtained at 9 and 7 months, respectively, after receipt of the MCC vaccine. The distribution of rSBA titers from an unvaccinated population are shown in Table 2. These data form a subset of the 1,689 serum samples from individuals of all ages in England and Wales taken between 1996 and 1999, prior to the national introduction of MCC vaccines, and tested for rSBA titers (19).

TABLE 3. Predicted vaccine efficacy and 95% CIs estimated for unvaccinated and vaccinated children with titers below the different SBA cutoffs 1 month after vaccination with the MCC vaccine measured by SBA

Group and cutoff	% Individuals with titers below cutoff		Predicted % vaccine efficacy (95% CI)
	Vaccinated	Unvaccinated	
Infants			
1:4	0.0	84.0	100 (86–100)
1:8	1.9	87.6	98 (85–100)
1:16	1.9	90.5	98 (86–100)
1:32	1.9	93.5	98 (84–100)
1:64	1.9	94.7	98 (86–100)
1:128	3.8	96.4	96 (85–99)
Toddlers			
1:4	4.3	84.3	95 (85–98)
1:8	8.6	87.3	90 (79–95)
1:16	12.9	91.4	86 (74–92)
1:32	17.1	92.9	82 (69–89)
1:64	27.1	95.1	71 (58–81)
1:128	32.9	96.6	66 (52–76)
Preschool children			
1:4	0.0	90.4	100 (95–100)
1:8	0.0	93.3	100 (95–100)
1:16	2.5	94.3	97 (92–99)
1:32	4.1	95.2	96 (90–98)
1:64	4.9	97.1	95 (89–98)
1:128	9.8	97.6	90 (83–94)

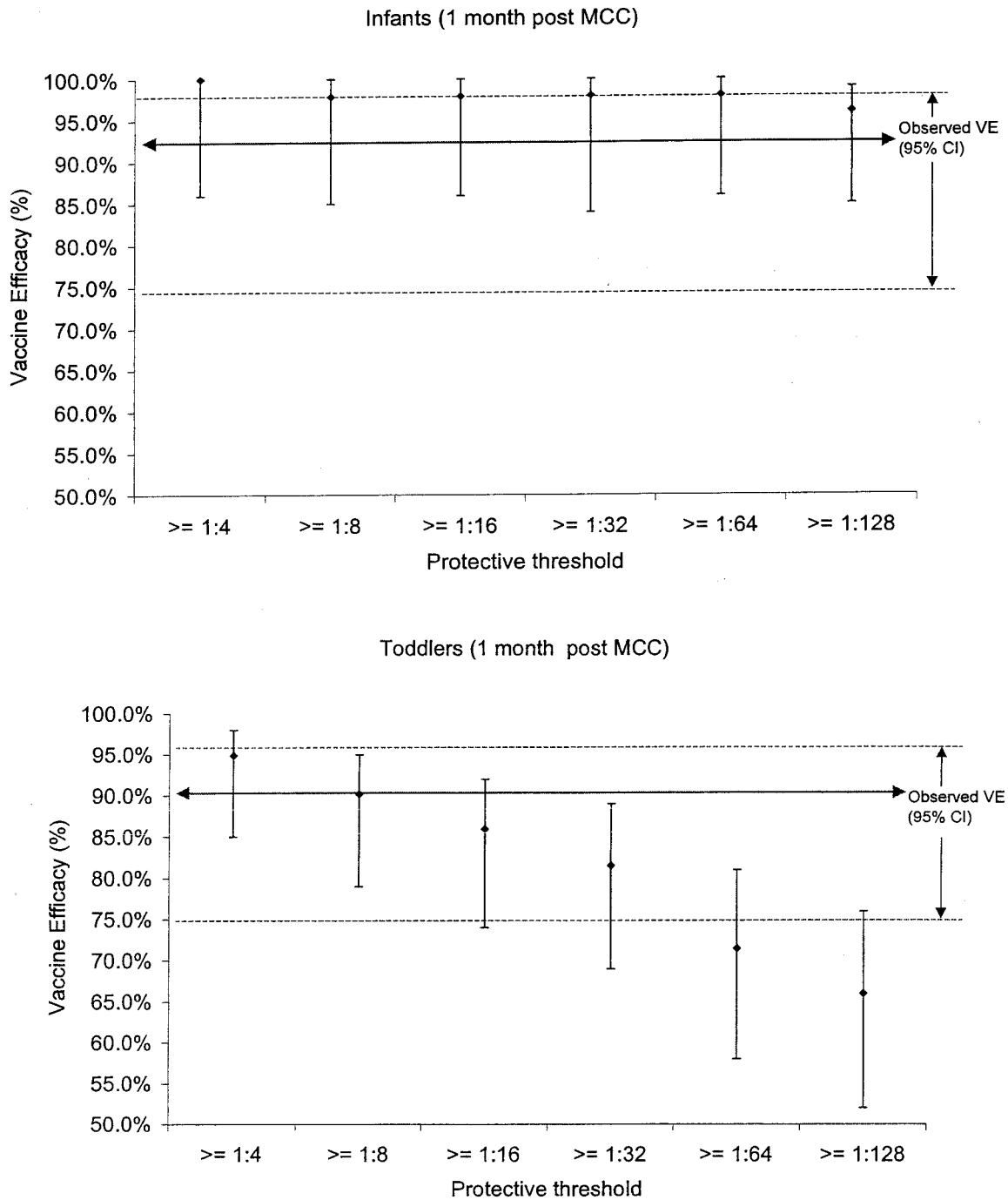


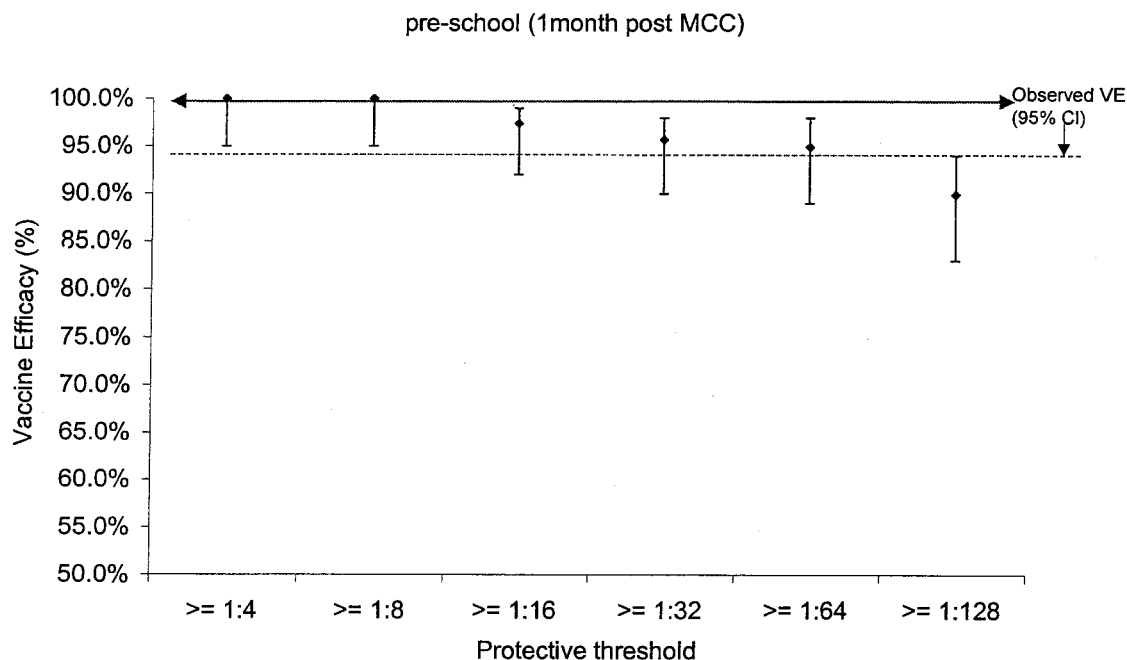
FIG. 1. Efficacy predicted on the basis of the proportions of unvaccinated and vaccinated individuals with titers below the different rSBA cutoffs measured 1 month following vaccination with the MCC vaccine.

## RESULTS

**Observed vaccine efficacy.** After partially vaccinated individuals were excluded, there were a total of 12 cases (4 vaccinated) of Men C disease in infants, 48 cases (7 vaccinated) of Men C disease in toddlers, and 27 cases (0 vaccinated) of Men C disease in preschool children. Observed efficacy estimates with 95% CIs for infants, toddlers, and preschool children were

92.5% (95% CI, 74.4 to 97.8%), 90.1% (95% CI, 74.9 to 96.1%), and 100% (95% CI, 93.3 to 100%), respectively.

**Vaccine efficacy predicted by different rSBA cutoffs at 1 month postvaccination with the MCC vaccine.** The efficacies predicted on the basis of the proportions of unvaccinated and vaccinated individuals with SBA titers below the different rSBA cutoffs measured 1 month following vaccination with the



MCC vaccine are shown in Table 3 and Fig. 1. For infants, the observed efficacy is lower than that predicted by all the cutoffs, although the CIs for the predicted efficacy all include the observed efficacy, so none of the cutoffs is inconsistent.

The toddler cohort provides the best data for discrimination between cutoffs because it includes the greatest numbers with postvaccination titers in the range of 4 to 64. The predicted efficacy is most consistent with the observed efficacy for the 1:8 cutoff. The results are inconsistent at cutoffs of 1:64 and 1:128 ( $P = 0.013$  and  $0.036$ , respectively).

For the preschool children, the predicted efficacy is most consistent with the observed efficacy at cutoffs of 1:4 and 1:8, although cutoffs between 1:16 and 1:64 are also consistent. However, the predicted efficacy at a cutoff of 1:128 is not consistent with the observed efficacy.

**Vaccine efficacy predicted by different rSBA cutoffs at 7 to 9 months postvaccination with the MCC vaccine.** By using the titers for infants at 9 months postvaccination as correlates of protection, all cutoffs significantly underestimated efficacy, so none is consistent (for the cutoff of 1:4,  $P = 0.03$ ) (Table 4 and Fig. 2). If the postvaccination titers at 7 months are used as correlates of protection for the toddlers, efficacy is also underestimated. The difference between the estimated and the observed efficacies for the toddler group is significant at all cutoffs above 1:4 (for the cutoff of 1:8,  $P = 0.040$ ; for the cutoff of 1:16,  $P = 0.049$ ; for the cutoff of 1:32,  $P = 0.008$ ).

## DISCUSSION

A serological correlate of protection for a vaccine may be based on the individual or the population (17). The individual-based correlate of protection involves the measurement of preexposure antibody levels in all vaccinated subjects and the relation of these levels to whether the subjects subsequently

develop disease, the objective being to identify a threshold level in the individual that predicts protection. This was the approach adopted in the 1960s by Goldschneider et al. (9) to establish the serological correlate of protection against Men C disease in those with natural immunity. By showing that only 3 of 54 (5.6%) military recruits who contracted Men C disease had a preexposure hSBA titer against Men C organisms  $\geq 4$  but that 444 of 540 (82.2%) of those who remained disease free had a preexposure hSBA titer  $\geq 4$ , a correlate of protection could be established at the individual level. In a vaccine efficacy trial, establishment of an individual correlate of protection requires postvaccination samples to be taken from all trial

TABLE 4. Predicted vaccine efficacy and 95% CIs estimated for unvaccinated and vaccinated infants and toddlers with titers below the different SBA cutoffs 7 to 9 months after vaccination with the MCC vaccine measured by SBA

Group and cutoff	% Individuals with titers below cutoff		Predicted % vaccine efficacy (95% CI)
	Vaccinated	Unvaccinated	
Infants			
1:4	26.0	84.0	69 (50–81)
1:8	46.0	87.6	47 (29–61)
1:16	52.0	90.5	43 (25–56)
1:32	58.0	93.5	38 (21–51)
1:64	66.0	94.7	30 (15–43)
1:128	72.0	96.4	24 (9–36)
Toddlers			
1:4	15.4	84.3	82 (68–90)
1:8	24.6	87.3	72 (57–82)
1:16	24.6	91.4	73 (59–82)
1:32	33.8	92.9	64 (49–74)
1:64	47.7	95.1	50 (35–61)
1:128	52.3	96.6	46 (32–57)

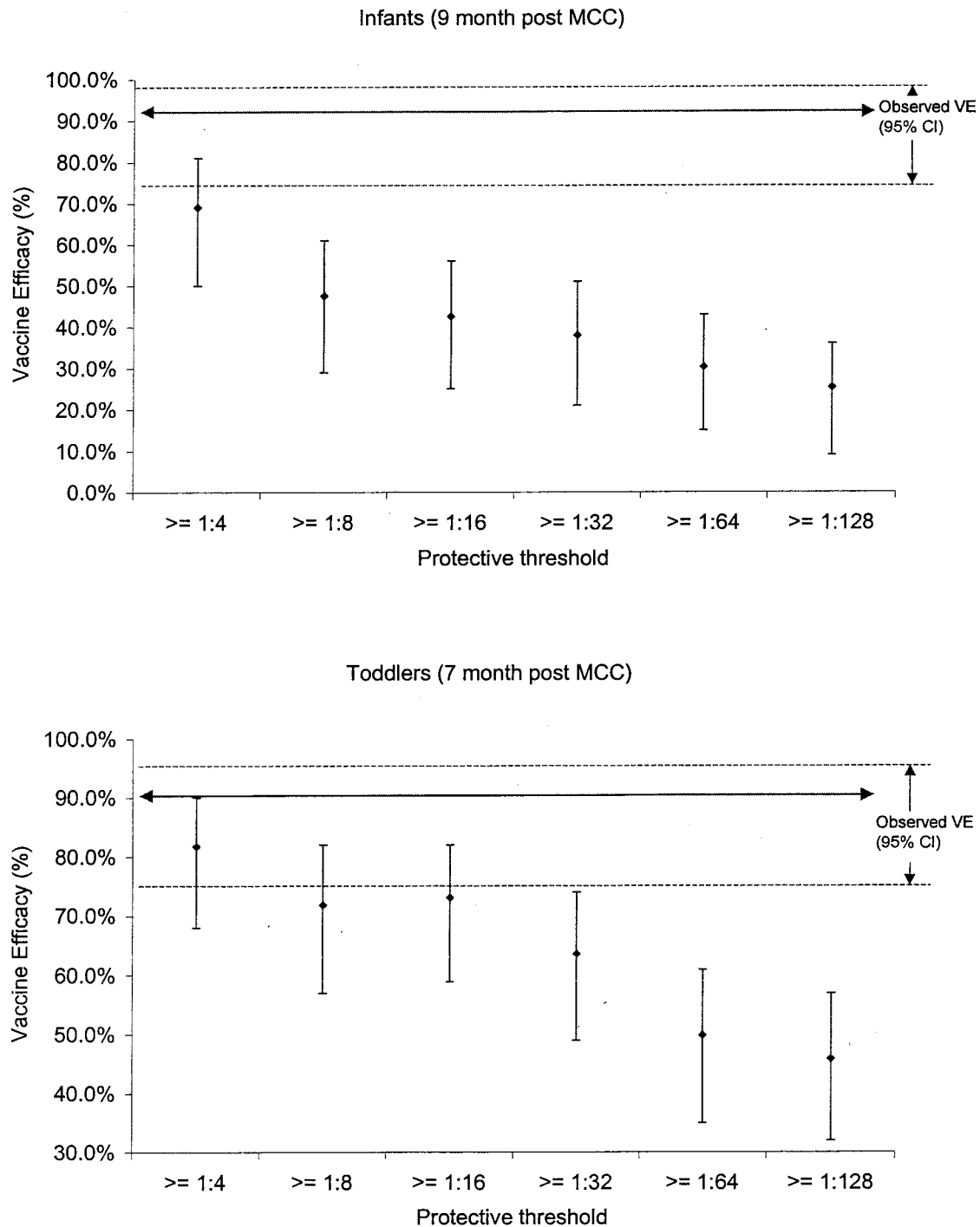


FIG. 2. Efficacy predicted on the basis of the proportions of unvaccinated and vaccinated individuals with titers below the different rSBA cutoffs measured 9 months (infants) or 7 months (toddlers) months following vaccination with the MCC vaccine.

subjects, which, in the context of a large meningococcal vaccine trial, would be difficult to accomplish. An alternative approach is to take early postexposure blood samples from trial subjects who have had a known contact with the disease and relate the disease outcome to antibody levels at the time of exposure. Again, this would not be feasible in the context of a meningococcal vaccine trial due to the very low secondary attack rates.

Reliance on a population-based correlate for the meningococcal vaccine or extrapolation of the individual disease-based hSBA correlate established by Goldschneider et al. (9) is therefore necessary.

The population-based correlate of protection requires the identification of the level of antibody that is achieved by the majority of a protected group (i.e., vaccinated individuals) and



not achieved by the majority of a susceptible group (i.e., unvaccinated individuals) (17). The level of protection correlated with that antibody level is the vaccine efficacy measured in a phase III trial or through postlicensure surveillance. For a population-based correlate it is necessary to measure immunogenicity only in a representative and statistically adequate sample of the vaccinated and unvaccinated populations in whom efficacy is measured. This was the approach adopted here.

Our study shows that the rSBA cutoffs for protection in the range of 1:4 to 1:64 at 1 month following vaccination with the MCC vaccine are consistent with the observed efficacy. The lower cutoffs of 1:4 and 1:8 are most consistent with the observed efficacy. The cutoff of 1:128 was shown to be inconsistent with the observed efficacy. These observations support the proposal that individuals with postvaccination rSBA titers between 8 and 64 with additional serological evidence of protection (namely, a fourfold rise in titer and/or evidence of induction of immunologic memory) are protected, despite the absence of an hSBA response with a titer  $\geq 4$ . It also confirms that an rSBA cutoff of  $\geq 128$  for presumption of protection is unnecessarily conservative.

Population correlates of protection may be supported by demonstrating an inverse relationship at the population level between the proportion of individuals in different age groups with titers above the putative protective level and the incidence of disease in that age group. This approach was originally adopted by Goldschneider et al. (9), who showed that the proportion of the population with hSBA levels  $\geq 4$  was inversely related to the incidence of Men C disease in that age group. We have shown a stronger inverse relationship for rSBA levels  $\geq 8$  than for levels  $\geq 128$  and the incidence of Men C disease in an unvaccinated population in the United Kingdom (19).

The results of the present study apply only to short-term efficacy, since the average intervals since vaccination were about 10 months for the toddlers and 8 months for infants. However, as rSBA levels in these groups decline rapidly within 7 to 9 months of immunization (14, 16), the average rSBA levels in the cohort in whom efficacy was measured will be substantially lower than the levels measured at 1 month postvaccination with the MCC vaccine. When the cutoffs for infants and toddlers at 9 and 7 months postvaccination were used, only the 1:4 level for toddlers was consistent with the observed efficacy; all other cutoffs significantly underestimated efficacy. This suggests that protection during the period when acute-phase postvaccination antibody levels have declined is less reliant on the actual SBA level at the time of exposure and more reliant on a rapid booster antibody response, reflecting invocation of immunologic memory. The presence of immunologic memory, as illustrated both by the response to a polysaccharide challenge and by the maturation of antibody avidity in the months following primary immunization with the MCC vaccine, has been shown after both a three-dose course in infants (16) and a single dose in toddlers (14).

Our data on the rSBA correlate of short-term protection for MCC vaccines is supported by data for the unconjugated C polysaccharide vaccine. In adults, the short-term efficacies of Men C polysaccharide vaccines have been estimated from a number of trials and are consistently about 90% (8, 1, 5). The proportion of young adults achieving rSBA titers  $\geq 8$  at 4 weeks after receiving a single dose of plain MCC polysaccharide has

been reported to be 94.4% (15), which is comparable to the efficacy estimates for adults. In children aged 24 to 36 months, the vaccine efficacy was reported to be 55% in a randomized trial in Brazil (18), whereas in 24- to 35-month-old children in the Murcia region of Spain, the proportion of responders with rSBA titers  $\geq 8$  was 58% (6). As expected, the efficacy of the unconjugated vaccine rapidly declines as SBA titers drop since the vaccine does not induce immunologic memory (5). Our study is the first indication that the conjugated vaccine will continue to offer protection by induction of immunologic memory when SBA levels have dropped.

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